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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner

Keith D. Hendricks

Group Art Unit

1761

Applicant

David Vincent Zyzak et al.

Application No.

10/606,137

Confirmation No.

3971

P&G Docket No.

9043MXL

Filed

June 25, 2003

For

METHODS FOR REDUCING ACRYLAMIDE IN

FOODS, FOODS HAVING REDUCED LEVELS OF

ACRYLAMIDE, AND ARTICLE OF COMMERCE

DECLARATION OF DAVID VINCENT ZYZAK SUBMITTED PURSUANT TO 37 C.F.R. § 1.131

Sir:

- I, David Vincent Zyzak, declare that:
- 1. I am a Senior Scientist at The Procter & Gamble Company ("P&G"), Winton Hill Business Center, 6300 Center Hill Avenue, Cincinnati, Ohio, 45224.
- 2. I understand that myself, Robert Alan Sanders, Marko Stojanovic, David Cammiade Gruber, Peter Yau Tak Lin, Maria Dolores Martinez-Serna Villagran, John Keeney Howie and Richard Gerard Schafermeyer ("Zyzak") are the named inventors of U.S. patent application Serial No. 10/606,137 (the "Zyzak '137 application"). I make this declaration in support of Zyzak's claim that the invention claimed in the Zyzak '137 application was made before the September 19, 2002 priority date of Elder et

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Appl. No. 10/606,137 November 28, 2006 37 CFR § 1.131 Declaration of David Vincent Zyzak Submitted With Response to Office Action

al.'s U.S. patent application 10/247,504 (the "Elder '504 application"), as well as before the September 11, 2002 publication of the Health Canada Letter entitled "Acrylamide in Food Update" (the "Health Canada Letter"). The Health Canada Letter was submitted to the US PTO in an IDS filed on June 14, 2006. I also understand that Zyzak is requesting that the U.S. Patent and Trademark Office declare an interference between the Zyzak '137 application, and the Elder '504 application.

- 3. I received a B.S. in Chemistry from Old Dominion University in 1989. I received a Ph.D. in Biochemistry from the University of South Carolina in 1995. My Ph.D. thesis was "Studies on the Maillard reaction: mechanism of the fructosamine assay, decomposition of Amadori adducts on protein, and reaction of 3-deoxyglucosone with arginine residues in protein."
- 4. Since I earned my Ph.D. in 1995, I have continuously been employed in research and development positions in the food industry. I am the author of numerous publications related to my research and development work in the food industry.
- From August 1995 until November 1997, I worked for Nestle in New Milford, Connecticut, as a Developmental Technologist and Process Flavor Chemist.
- 6. From November 1997 until September 1999, I worked for Takasago Institute, a flavors and fragrances company located in Rockleigh, New Jersey. My position at Takasago was Senior Scientist.
- 7. In September 1999, I started working for P&G in Cincinnati, Ohio. When I joined P&G, my position was Scientist in P&G's Food and Beverage Analytical/Microbiology Division. In September 2000, I was promoted to Senior Scientist. In 2002, the name of the Food and Beverage Analytical/Microbiology Division was changed to Snacks and Beverage Analytical/Microbiology. In 2004, the name was changed again to Household Care Analytical. Today I am a Senior Scientist in P&G's Household Care Analytical Division. I am also the Coordinator of Coffee Analytical Support. During my employment at P&G, I have worked continuously in research and development related to snack food products.
- 8. I conducted an experiment entitled "Use of Asparaginase to decrease acrylamide formation in cooked foods" (the "Experiment"). The Experiment was conducted at the Winton Hill Business Center, a P&G facility in Cincinnati, Ohio.

- I recorded the details of how I conducted the Experiment on pages
 and 3 in my P&G Lab Notebook #WHS 2688.
- 10. A true and correct copy of the cover, instruction sheet, and pages 2 and 3 of my P&G Lab Notebook #WHS 2688 I attached hereto as Exhibit A. The dates on Exhibit A have been blacked out, but all of the dates are before September 10, 2002.
- 11. In the Experiment's first step, baking potatoes were boiled for two hours. The potatoes were then peeled and mashed with a fork.
- 12. Next, 100 grams of the mashed potatoes that were prepared in the Experiment's first step were mixed with 100 grams of distilled and de-ionized water, and the resulting mixture was homogenized until it was uniform and no lumps were visible.
- 13. Next, four samples were prepared. Each sample consisted of 30 grams of the mixture described above in paragraph 12, mixed with 30 grams of distilled and de-ionized water. Each sample was placed in an eight ounce glass jar, and the four samples were labeled A1, A2, E1 and E2, respectively.
- 14. A solution was prepared consisting of 500 units of asparaginase dissolved in 1.0 milliliter of distilled and de-ionized water. One unit of asparaginase is defined as the amount of asparaginase that will liberate 1.0 micromole of NH₃ from L-asparagine per minute at 37° C and a pH of 8.6. The asparaginase I used was ordered before September 10, 2002, from VWR, a vendor that arranges ordering and shipping of scientific products within P&G. A true and correct copy of the email I sent to VWR asking that they order the asparaginase from Sigma-Aldrich Inc. is attached hereto as Exhibit B. A true and correct copy of the Sigma-Aldrich Inc. invoice for the asparaginase order is attached hereto as Exhibit C. The dates on Exhibits B and C have been blacked out, but all of the dates are before September 10, 2002.
- 15. 100 microliters of the asparaginase solution described above in paragraph 14 was added to the jar labeled E1, and 100 microliters of the same solution was added to the jar labeled E2. No asparaginase solution was added to the jars labeled A1 and A2, as those jars served as controls.
- 16. Next, the four samples described above in paragraph 15 were allowed to stand at room temperature for 30 minutes with occasional stirring to allow the asparaginase in the jars labeled E1 and E2 to react with the asparagine in the potatoes.

- 17. The four samples described above in paragraph 16 were then micro-waved for two minutes to deactivate the asparaginase in the jars labeled E1 and E2.
- 18. The four samples described above in paragraph 17 were then micro-waved in two minute sessions until the samples were cooked. This required four two minute sessions, for a total of eight minutes.
- 19. The four samples described above in paragraph 18 were then sent to P&G's Foods and Beverages Analytical/Microbiology lab for analyses of the acrylamide, asparagine and aspartic acid contents of the samples. Deborah K. Ewald performed the acrylamide testing, and Janice N. Batchelor performed the asparagine and aspartic acid testing.
- 20. I received the results of the acrylamide analysis from Deborah Ewald. These results were tabulated in a spreadsheet, a true and correct copy of which is attached hereto as Exhibit D. I also recorded these results on page 3 of my Lab Notebook #WHS 2688 (Exhibit A). The dates on Exhibit D have been blacked out, but all of the dates are before September 10, 2002.
- 21. The lab results show that for the jars labeled A1 and A2 (the two samples that were not treated with the asparaginase solution), the acrylamide levels were 21,605 and 20,543 parts per billion ("ppb") respectively. For the jars labeled E1 and E2 (the two samples that were treated with the asparaginase solution), the acrylamide levels were 385 and 164 ppb, respectively.
- 22. The results described above in paragraphs 20 and 21 demonstrate that, in the case of the samples in the jars labeled E1 and E2, the addition of asparaginase to the mashed potato mixture caused the acrylamide levels to be reduced by over 98% after cooking, as compared to the levels of acrylamide in the untreated samples in the jars labeled A1 and A2.
- 23. I received the results of the asparagine and aspartic acid analyses from Janice N. Batchelor. Those analyses were performed before September 10, 2002. A true and correct copy of those results is attached hereto as Exhibit E. I also recorded those results on page 3 of Lab Notebook #WHS 2688 (Exhibit A).
- 24. The lab results I received show that for the jars labeled A1 and A2 (the two samples that were not treated with the asparaginase solution), the asparagine levels were 1131.0 and 1041.6 parts per million ("ppm"), respectively. For the jars

labeled E1 and E2 (the two samples that were treated with asparaginase solution), the asparagine levels were 129.5 and 195.5 ppm, respectively. For the jars labeled A1 and A2 (the two samples that were not treated with the asparaginase solution), the aspartic acid levels were 189.2 and 178 ppm, respectively. For the jars labeled E1 and E2 (the two samples that were treated with the asparaginase solution), the aspartic acid levels were 1307 and 1826.5 ppm, respectively.

- 25. The results described above in paragraphs 23 and 24 demonstrate that, in the case of the samples in the jars labeled E1 and E2, the addition of asparaginase to the mashed potato mixture caused the asparagine levels to be reduced by over 85% after cooking, as compared to the levels of asparagine in the untreated samples in the jars labeled A1 and A2.
- 26. The results described above in paragraphs 23 and 24 demonstrate that, in the case of the samples in the jars labeled E1 and E2, the addition of asparaginase to the mashed potato mixture caused the aspartic acid levels to be increased by over 753% after cooking, as compared to the levels of aspartic acid in the untreated samples in the jars labeled A1 and A2.
- 27. I explained the Experiment, its results, and the significance of the results, to Dr. Kwan Y. Lee, a Principal Scientist in P&G's Food and Beverages Analytical/Microbiology Division in Cincinnati, Ohio. I also showed him pages 2 and 3 of my P&G Lab Notebook #WHS 2688 (Exhibit A). Dr. Lee signed and dated page 2 of my entry in Lab Notebook #WHS 2688. He also dated page 3, but did not sign it. I believe that Dr. Lee's failure to sign page 3 was an oversight. The dates on Exhibit A have been blacked out, but all of the dates are before September 10, 2002.
- 28. I understand that claim 1 of the Zyzak '137 application reads as follows:

A method for reducing the level of asparagine in a food material, comprising adding an asparagine-reducing enzyme to the food material before heating.

29. The Experiment discussed above in paragraphs 11 through 19 corresponds to claim 1 of the Zyzak '137 application. In the Experiment, I reduced the level of asparagine in mashed potatoes (a food material) by adding asparaginase (an

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asparagine-reducing enzyme) to a mixture of mashed potatoes and water before I heated the mixture in a microwave oven.

30. I understand that claim 10 of the Zyzak '137 application reads as follows:

A method for reducing the level of acrylamide in food, comprising:

- adding an asparagine-reducing enzyme to a food material, wherein said food material comprises asparagine;
- 2) optionally mixing the enzyme with the food material;
- allowing a sufficient time for the enzyme to react with asparagine;
- optionally deactivating or optionally removing the enzyme;
 and
- 5) heating the food material to form the finished food product.
- 31. The Experiment discussed above in paragraphs 11 through 19 corresponds to claim 10 of the Zyzak '137 application. In the Experiment, I mixed a solution containing asparaginase (an asparagine-reducing enzyme) with a mixture of mashed potatoes (a food material that contains asparagine) and water. I then allowed the mixture of asparaginase solution, mashed potatoes and water to sit for 30 minutes, which was sufficient time for the asparaginase to react with asparagine. I then deactivated the asparaginase by micro-waving the mixture for two minutes. I then heated the mixture for a total of eight minutes in a micro-wave oven, at which point it was cooked. I then had the cooked material tested for acrylamide. The acrylamide levels were more than 98% lower than acrylamide levels in the control runs that were not treated with asparaginase solution. Immediately after completing the experiments discussed in paragraphs 11 through 19, and receiving the results discussed in paragraphs 20 through 24, I worked diligently with my co-inventors and a patent attorney employed by the Procter & Gamble Company, to further reduce the present invention to practice and to prepare and file U.S. patent application Serial No. 10/606,137.

32. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the Zyzak '137 application or any patent issuing therefrom.

Application No. 10/606,137 P&G Docket No. 9043MXL

EXHIBIT A

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P&G Restricted
subject like of Asparinginese to decreve culylando formation in lakeli
Background Cur data suggest that apparagine is the source of
acrylimide formation in hented potatoes (and possibly in all foods).
If we use the enzyme appropriate, which convocts asperagine to aspects acid, we should be able to drawe acrylamide
formation in hasted potenties.
Beagents / Supplies:
1 Mashed potatoes - made by beiling baking patentees obtained
from local supermerket, for 2 hrs. The builed potenties
are de-peeled and mashed with a ferk. (2) Asparaginase
Sigma A 2925 (See with dissolved in 1.0 ml
distilled and dejonized water.
One unit definition one unit will liberate no unite of
NH3 from L-apparegine per minute at pH 8.6 at 37°C
Trial is labeled as 3:6 mg Selid and protein content is 40%
3) Panasenie Microwave Midel NN-55488A
Procedure to prepare mashed potato Sturry: (D) Take 1000 of mashed potatoes.
190 Add 100 g of distilled and decenized water
3) Homogenize until uniform and no Longs are visible.
Experiments:
O Take 300 of mashed potate sturry and place in 800 splass
<u>jar.</u>
(3) Add 30g distilled and deirnized water.
This was done to prepare 4 jars labeled A1, A2, E1, + E2
3 To jars labeled E1 and E2, add 100 at of the
asparsginuse solution. This is equivalent to 50 units
of approximately 1.44 ag propers.
Worker's Signature Date
Corroborating Witness Curan Date Date

•	DateP&G Restricted	3
	subject Asparagine continued from p. 2	
] .	@ Let samples Stand at room temporature for	
_	30 min with ocassismal stirring /swirling every	
4		
	5 To deactivate enzyme: Microwave samples for	
- ·	d min on high setting. Treat samples without	+
4	asperaginase (A1 + A2) the same.	
-	[Microwaving was done in pairs Alana together; 6	1+62
4	together	
-1.		
_	6 Continue to microwave in 2 min sessions	until
	sturry is dried. This took 4 sessions and	الم
	4 Samples (A), A2, A E1, E2) turned reddish brown	
·	here was no apparent difference between	Al As
	El and Ea in color or degree of drynes	s. The
- }	microwave drying appeared to work well. The	droma
-	Al, AZ, E1 + EZ were very similiar - vegetable pr	dein
	like similiar to a mild Hydrolyged Plant Protein (HPP)
- ·	with potato undertones.	
1	O Submit sumples for acrylamide analysis and	
]	asparagine andysis	
	Manage Mars)	/ 1
	2 1. 0.2. 0 1 1 ()	(ppm)
4	A1 21,605 1131.0	189.2
1	A2 26,543 1041.6	178.0
	5)	1307.0
		1826.5
		10 00,5
	Results:	
	0 98.7 % inhibition of acculanite formation with asp	oraginse.
		<u> </u>
	Worker's Signature	
ı	Complementing Witness	
	Date	

Application No. 10/606,137 P&G Docket No. 9043MXL

EXHIBIT B

David Zyzak-DV

To: Special Vwr-IM/PGI

11:06 AM

CC: Subject: order

I would like to order the following chemical from sigma 1-800-325-3010.

item **Asparaginase** catalog Number A 2925

quantity

size 500 units price 82.35 (each)

needed by Tuesday

Please mail to:

Debble Ewald (Room F1B30) P&G 6071 Center Hill Ave. Cincinnati, OH 45224

Please charge to my AMEX: 3787 325567 41009

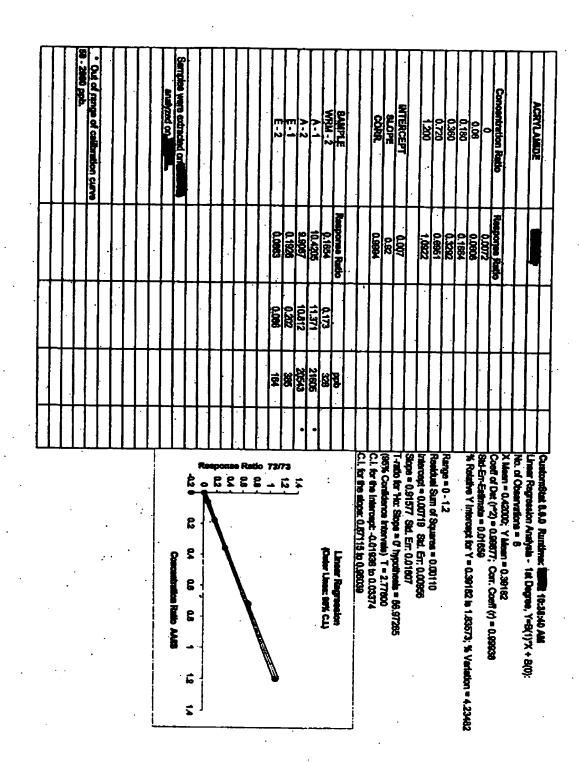
Thanks, David Zyzak

Application No. 10/606,137 P&G Docket No. 9043MXL

EXHIBIT C

Application No. 10/606,137 P&G Docket No. 9043MXL

EXHIBIT D



Application No. 10/606,137 P&G Docket No. 9043MXL

EXHIBIT E

CLASS-VP V 5.03 External Standard Report

Page 1 of 1 (6)

thod Name:

C:\CLASS-VP\METHODS\Asparagine extended.met

Sequence Name:

C:\CLASS-VP\SEQUENCE\GLUCOSAMINE\

Data Name:

C:\CLASS-VP\DATA\SORBEN\@LUUGUU

Sample ID;

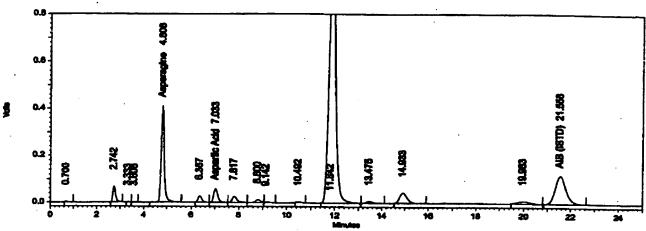
Al 131 Sangle Set

User:

System

Acquired: Printed:

6:07:35 PM 6:34:07 PM



Sample Amount:

Multiplier Factor: 1

Fluorescence Detector

(Ex:260лm.

Em:313nm)

Pk #	Name	Retention Time	Area	ISTD concentration	Units
5	Asparagine	4.81	3949539	1131.042	pom
7	Aspartic Acid	7.03	696440	189.169	ppm
16	AIB (ISTD)	21.56	3765554	0.000	ppm

Page 1 of 1 (7)

CLASS-VP V 5.03

External Standard Report

shod Name:

C:\CLASS-VP\METHODS\Asparagine extended.met

C:\CLASS-VP\SEQUENCE\GLUCOSAMINE\

Data Name:

C:\CLASS-VP\DATA\SORBEN

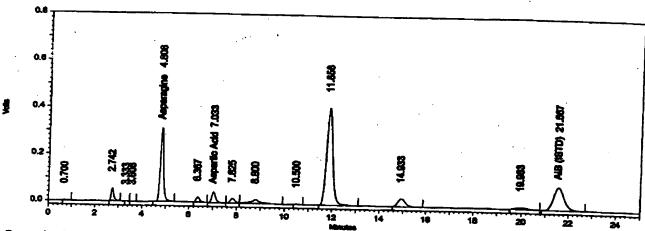
Sample ID;

2 14 Sample Set

User: Acquired: System

Printed:

6:34:08 PM 7:00:41 PM



Sample Amount:

Multiplier Factor:

Fluorescence Detector (Ex:260nm, Em:313nm)

Pk#	Name	Retention Time	Area	ISTD concentration	Units
	Asparagine Aspartic Acid AIB (ISTD)	4.81 7.03 21.57	2949846 532709 3052920	1041.552 177.953 0.000	ppm ppm

CLASS-VP V 5.03

External Standard Report

Page 1 of 1 (8)

Chod Name:

C:\CLASS-VP\METHODS\Asparagine extended.met

equence Name:

C:\CLASS-VP\SEQUENCE\GLUCOSAMINE\

Data Name:

C:\CLASS-VP\DATA\SORBEN\

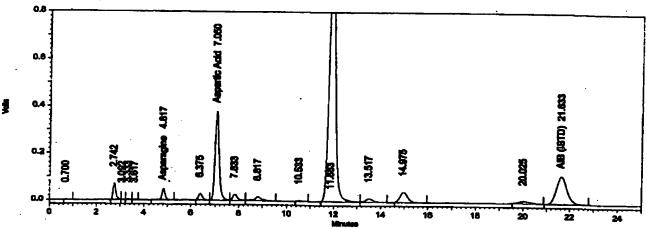
Sample ID;

El 1st Sample Set -Asparquase - Heartel

User: Acquired: System

Acquired:

7:00:42 PM 7:27:20 PM



Sample Amount:

Multiplier Factor:

Fluorescence Detector (Ex:260nm,

Em:313nm)

	Name	Retention Time	Area	ISTD concentration	Units
6	Asparagine	4.82	461708	129.529	ppm
	Aspartic Acid	7.05	4562675	1307.031	ppm
16	AIB (ISTD)	21.63	3717360	0.000	ppm

CLASS-VP V 5.03

External Standard Report

Page 1 of 1 (9)

thod Name:

C:\CLASS-VP\METHODS\Asparagine extended.met

Sequence Name:

C:\CLASS-VP\SEQUENCE\GLUCOSAMINE

Data Name:

C:\CLASS-VP\DATA\SORBEN\

Sample ID;

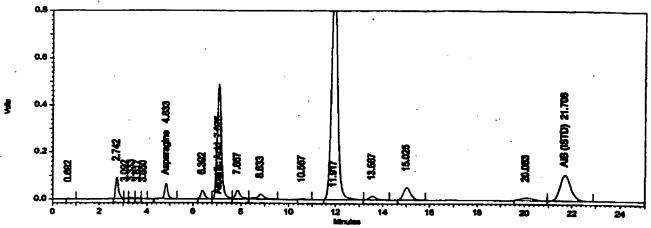
E2 1st Sample Set

User:

System

Acquired: Printed:

7:27:21 PM 7:54:01 PM



Sample Amount:

Multiplier Factor:

Fluorescence Detector (Ex:260nm, Em:313nm)

Pk#	Name	Retention Time	Area	ISTD concentration	Units
7	Asparagine	4.83	641523	195.516	pom
9	Aspartic Acid	7.08	5932050	1826.512	
17	AIB (ISTD)	21.71	3465326	0.000	ppm